Isoetes yunguiensis (Isoetaceae), a New Basic Diploid Quillwort from China

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ABSTRACT. *Isoetes yunguiensis*, a basic diploid species with a chromosome number of $2n = 22$, is described and illustrated. This species occurs on the Yunnan–Guizhou Plateau in southwest China. It is similar to *I. japonica* in its appearance and emergent aquatic habit. Herbarium specimens of *I. yunguiensis* previously have been misidentified as *I. japonica*. *Isoetes yunguiensis* differs from *I. japonica* in megaspore and microspore morphology and chromosome number.

Key words: China, East Asia, Isoetaceae, *Isoetes*, quillwort, Yunnan–Guizhou Plateau.

Two basic diploid species ($2n = 22$), *Isoetes hypsophila* Handel-Mazzetti and *I. taiwanensis* DeVol, one tetraploid species ($2n = 44$), *Isoetes sinensis* Palmer, and one hexaploid species ($2n = 66$), *I. japonica* A. Braun, have been reported from China (Diao, 1990; DeVol, 1972). Over the last four years, field, herbarium, and laboratory studies have been conducted on the genus *Isoetes* in the vast area of mainland China, where quillworts are increasingly threatened with extirpation due to ongoing anthropogenic changes. During the course of these studies, specimens collected from the Yunnan–Guizhou Plateau in southwest China that superficially resembled and had been identified as *I. japonica* were found in herbaria. Study of these herbarium specimens and live specimens from the field revealed that they were different from *I. japonica* in spore morphology and chromosome number. These specimens represent a previously undescribed species of *Isoetes*.

Isoetes yunguiensis Wang Q. F. & W. C. Taylor, sp. nov. TYPE: China. Guizhou: Pingba County, Shashi Valley, plants in *Juncus bufolius–Eriocaulon schochianum* marsh, along a stream originating from the Shashi Valley Reservoir, ca. 4 km E of Pingba Town, 9 Aug. 2001, *Wang Q. F., Liu X. & Yang X. L. WH2001166* (holotype, WH). Figures 1, 2.

Planta aquatica, submersa vel emergens ex caudice trilobo. Folia 20 ad 70, viridia, 10–52 cm longa, patentissima, basi dilatata et complanata, marginibus membranacea; ligula triangulata, cuspidata, 1.0–2.5 mm longa, 1.5–3.0 mm lata. Sporangium basale, ovale, 2–7 mm longum, 1.5–4.5 mm latum; velum brevissimum, fibris peripheric is destitutum. Megasporae albae, 340–430 μm diametro, cristate-reticulatae. Microsporae pallide canae in massa dispositae, ellipticae, 20–25 μm longae, laeves vel granulatae. Chromosomatum numerus $2n = 22$ (Fig. 2I).

Plants aquatic, submersed or emergent. Rootstock 3-lobed. Leaves spirally arranged, widely spreading, 10–52 cm long, ca. 2.5 mm wide at mid length, in tufts of 20 to 70, flattened on upper side, rounded on lower side (Fig. 1C), base expanded with membranous margins, peripheral fibers absent. Ligule triangular, cuspidate, 1–2.5 × 1.5–3.0 mm. Velum rudimentary, covering only the distal edge of the sporangium. Sporangia basal, oval, 2–7 × 1.5–4.5 mm (Fig. 1B). Megaspores gray when wet, white when dry, 340–430 μm diam. (mean = 390 μm), with cristate-reticulate ornamentation (Fig. 2A–E). Microspores gray in mass, elliptic, 20–25 μm long (mean = 23 μm), laevigate to granulate (Fig. 2F–H). Some sporangia contain both megaspores and microspores. The megaspores in these mixed sporangia are slightly smaller than those

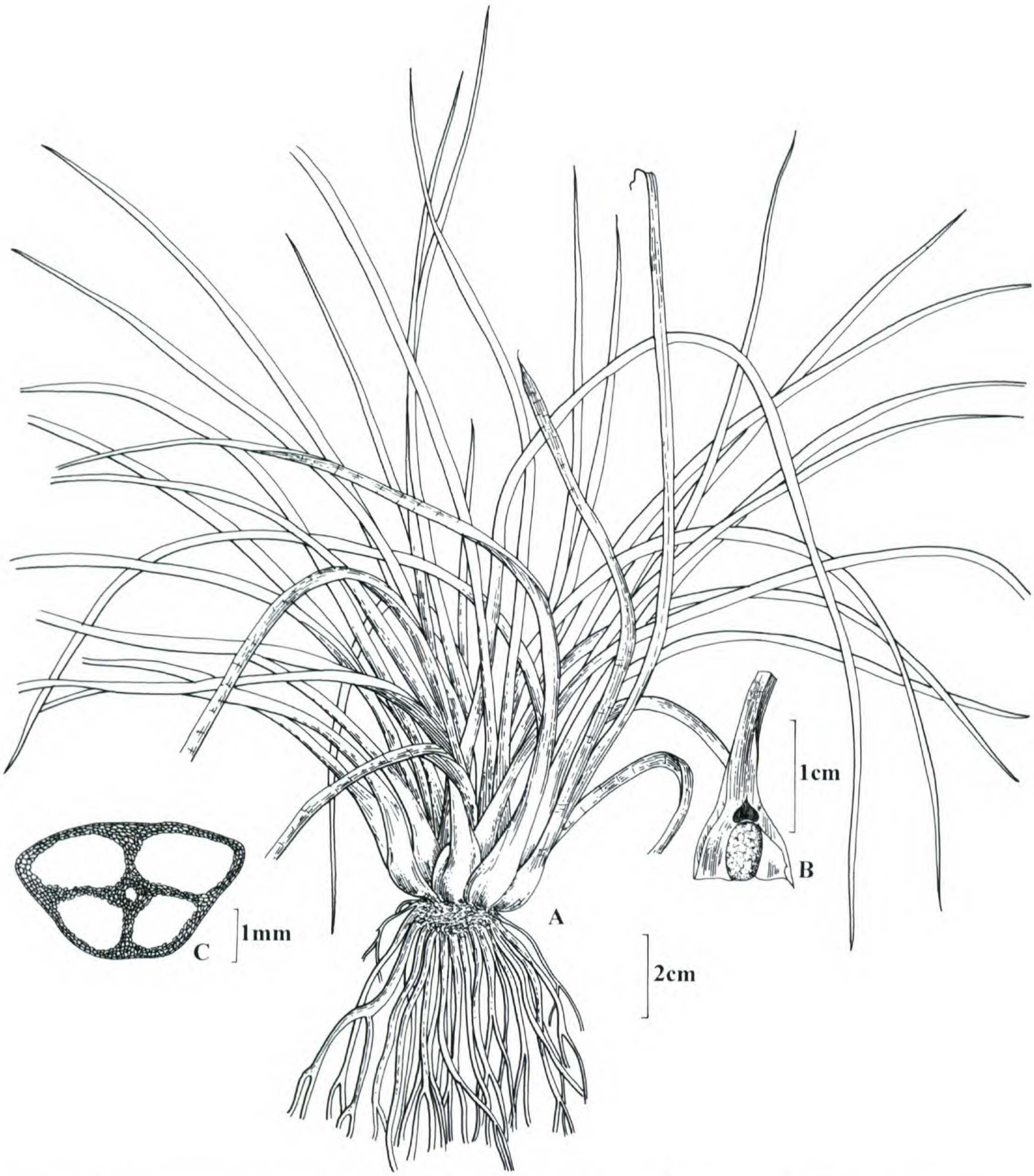

Figure 1. *Isoetes yunguiensis* Wang Q. F. & W. C. Taylor. —A. Habit. —B. Adaxial view of leaf base. —C. Transsection of leaf at mid length. Scale bar: A = 2 cm; B = 1 cm; C = 1 mm.

found in normal megasporangia. Chromosomes: $2n = 22$.

Distribution. In China, Kunming City and Xundian County of Yunnan Province; Pingba County and Guiyang City of Guizhou Province.

The specific epithet for this species is derived from the names Yunnan and Guizhou, the provinces from which the new species has been collected. These two provinces constitute the main section of the Yunnan–Guizhou Plateau in the upper reaches of the Yangtze River in southwest China.

Spore morphology. Spores of *I. yunguiensis* were obtained from specimens collected at the Shashi Valley and Shuangshao Village localities. Samples were prepared for scanning electron microscopy following the methods of Huang et al. (1992). Observations, measurements, and photomicrographs were made using a Hitachi S-800

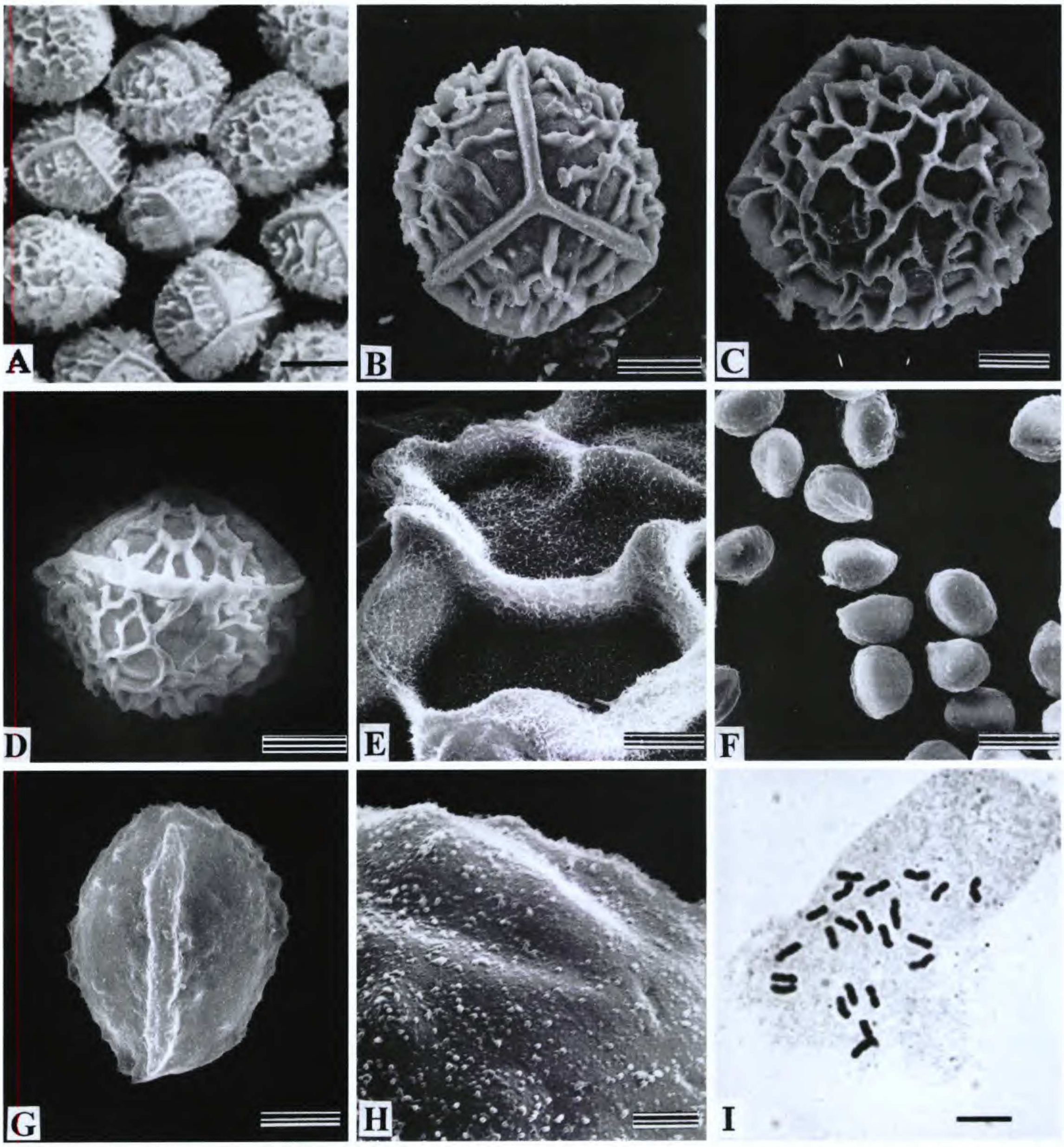

Figure 2. *Isoetes yunguiensis* Wang Q. F. & W. C. Taylor. —A. Megaspores. —B. Proximal view of megaspore. —C. Distal view of megaspore. —D. Equatorial view of megaspore. —E. Megaspore surface. —F. Microspores. —G. Microspore. —H. Microspore surface. —I. Somatic chromosomes in mitotic root tip squash (*Chu W. M. & He Z. R. 29306* (MIL)). Scale bar: A = 200 μm; B & D = 75 μm; C = 50 μm; E & F = 15 μm; G = 4 μm; H = 1 μm; I = 10 μm.

scanning electron microscope and with Olympus BX60 and SZX12 research microscopes. Megaspores of *I. yunguiensis* have a cristate, irregularly reticulate texture with the crests of the ridges uneven (Fig. 2 A–E). These megaspores are 360–450 μm diam. ($\bar{x}$ = 390 μm diam., N = 30). In contrast, the megaspores of *I. japonica* have a more regularly reticulate texture with the crests of the ridges more uniform (Takamiya, 1999: 124, fig. 10B). The megaspores of *I. japonica* are larger, 390–550 μm diam. ($\bar{x}$ = 500 μm diam., N = 30).

The cristate-reticulate megaspores of *I. yunguiensis* clearly differ from the other basic diploid species in East Asia. *Isoetes asiatica* (Makino) Makino has echinate megaspores (Takamiya et al., 1997), *I. hypsophila* has laevigate megaspores (Palmer, 1927), and *I. taiwanensis* has rugulate-reticulate megaspores (Huang et al., 1992; Britton &

Brunton, 1991). The tetraploid *I. sinensis* has megaspores with a cristate texture (Takamiya et al., 1997; Takamiya, 1999).

Microspores of *I. yunguiensis* have a laevigate to verrucate texture (Fig. 2F, G). At higher magnifications, using the scanning electron microscope, the microspores are seen to have a granulate texture (Fig. 2H). In texture, the microspores of *I. yunguiensis* are similar to *I. japonica* (Takamiya, 1999: 124, fig. 10E), but *I. yunguiensis* microspores are smaller than *I. japonica* microspores. The microspores of *I. yunguiensis* are 20–25 μm long and average 23 μm long. In contrast, the microspores of *I. japonica* are 28–34 μm long and average 31 μm long.

Although the megaspores and microspores of *I. yunguiensis* differ in size from those of *I. japonica*, *I. yunguiensis* is similar to *I. japonica* in its appearance and emergent aquatic habit. They are much alike in corm, leaf, sporangium, and velum characters. Both species occur in marshes, ponds, and streams. Early descriptions of *Isoetes* species were based on habit and habitat, and this could be the reason all of the *I. yunguiensis* plants deposited in different herbaria in China were identified as *I. japonica*.

Cytology. Plant materials for chromosome counts were collected from the Shashi Valley and Shuangshao Village localities and cultured in pots at the Botanical Garden, College of Life Sciences, Wuhan University, and in the Botany Department at the Milwaukee Public Museum. Root tips were prepared for observation of somatic chromosomes following the methods of Takamiya et al. (1994) and Taylor and Luebke (1988). Chromosome counts from 70 cells in root tip squashes from 14 individuals showed that *I. yunguiensis* is a basic diploid with the chromosome number of $2n = 22$ (Fig. 2I). The discovery of this basic diploid species brings the total of basic diploids in East Asia to four, including *I. asiatica*, *I. hypsophila*, and *I. taiwanensis*, out of a total of about eight species.

Isoetes yunguiensis is the only East Asian, basic diploid species with a vegetative morphology similar to the hexaploid *I. japonica*. Therefore, one could hypothesize that *I. yunguiensis* is involved in the evolution of *I. japonica*. It is possible that *I. japonica* is an autohexaploid, arising through autopolyploidy in the basic diploid *I. yunguiensis*. Alternatively, it is possible that *I. japonica* is an allohexaploid arising through allopolyploidy involving a cross between *I. yunguiensis* and a tetraploid species to produce a sterile triploid hybrid that doubled its chromosome number. Relationships among the basic diploid and polyploid *Isoetes* species distributed in East Asia, such as those between the basic diploid *I. yunguiensis* and the hexaploid *I. japonica*, need to be investigated by cytological and molecular studies.

Ecology. The streams, marshes, and ponds in which *I. yunguiensis* grows belong to the river systems of the Yangtze River on the Yunnan–Guizhou Plateau at an altitude of 1200–1900 m. Water pH value here is about 6.3. As with most quillworts, *I. yunguiensis* is easy to overlook, for it blends in with associated species such as *Juncus bufonius* L., *Leersia hexandra* Swartz, *Rotala rotundifolia* (Buchanan-Hamilton) Koehne, *Mariscus umbellatus* Vahl, *Hippuris vulgaris* L., *Eriocaulon schochianum* Handel-Mazzetti, and *Hypericum japonicum* Thunberg ex Murray.

Megaspores of *I. yunguiensis* mature from the middle of June to the end of September. Microspores develop from the end of June to the middle of October. By the end of October most sporophylls have decayed, and only a few small leaves that lack sporangia remain green during the winter. A similar overwintering condition is found in *I. sinensis*.

Like all other quillworts in China, *I. yunguiensis* is a rare and endangered species. To date it has been found at only four sites. Field investigations have revealed that two of these populations have been decimated. Of the remaining two sites, the Shuangshao population covers about 100 square meters and contains less than 100 plants, and the Pingba population covers about 400 square meters and holds 200 to 300 individuals. Pollution and increasing urbanization in China continue to raise the risk of extinction for this rare species. Searches for more field localities as well as conservation studies on this species are urgently needed.

Paratypes. CHINA. **Guizhou:** Pingba County, Shashi Valley, *Wang P. S. 75043, 75425* (CDBI, HGAS); Guiyang City, Wangwu, *Peng H., Wang P. S. & Zhao P. 79060* (HGAS). **Yunnan:** Kunming City, Heilongtan, *Chu W. M. 3636* (PYU, WH); Xundian County, Tianshengqiao, *Song Z. H. 80902* (PYU, WH); Kunming City, Shuangshao Village, swamp near reservoir, *Chu W. M. & He Z. R. 29306* (MIL, PYU).

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